Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Self-assembling properties of all γ-Cyclic Peptides containing sugar amino acids residues

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SUPPORTING INFORMATION

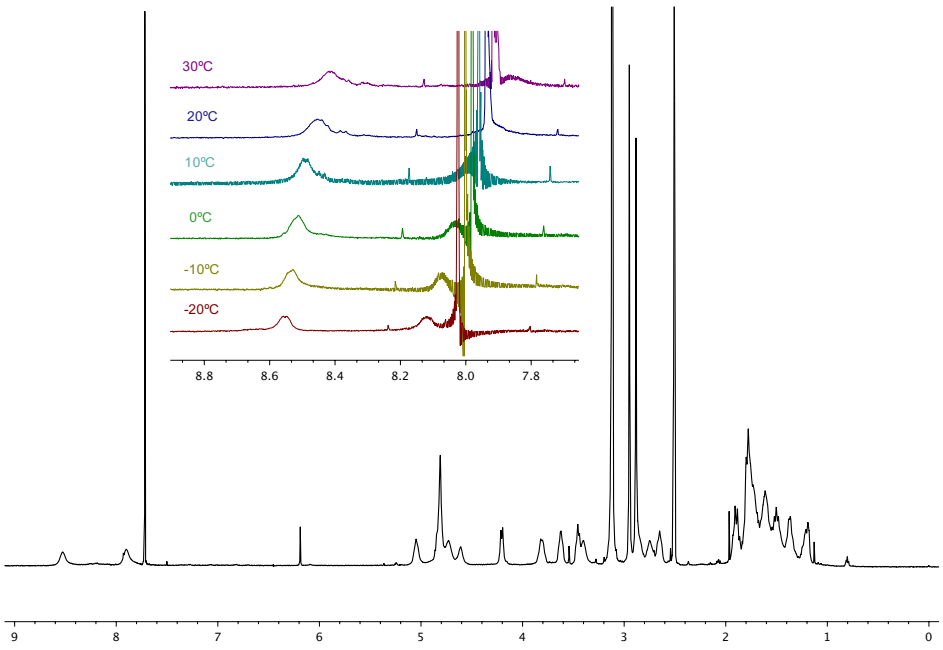


Figure 1SI: ^{1}H NMR spectra of **γ-CP2** in DMSO/$CDCl_3$ (3:7) at 298K that adopt the flat conformation, in the inset the spectra of amide region (8.90-7.70 ppm) at different temperatures (253-303K) are showed.

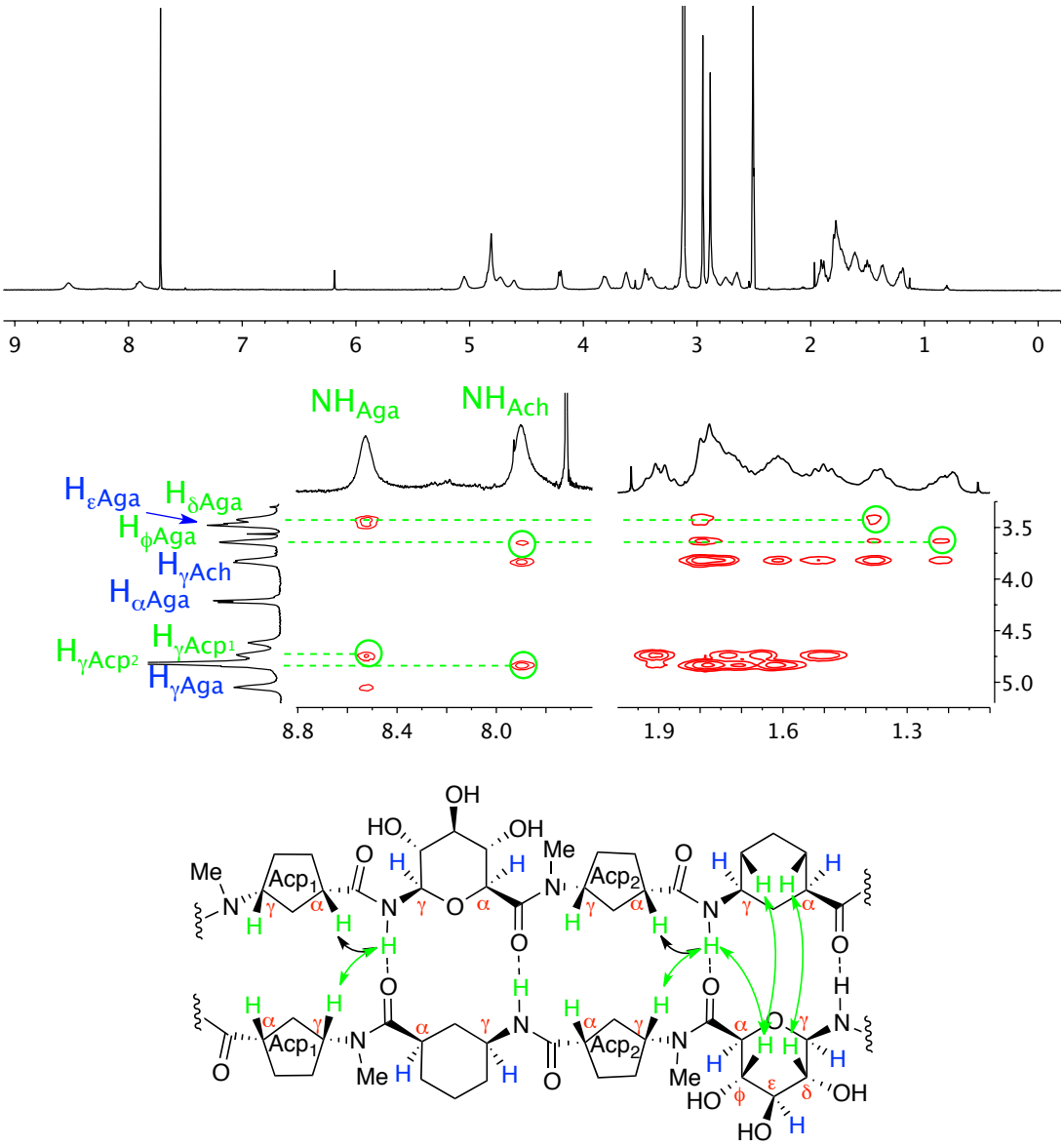


Figure 2SI: top ^{1}H NMR spectrum of **γ-CP2** in DMSO/$CDCl_3$ (3:7) at 298K. **Bottom** NOESY spectrum showing the nOe cross-peaks between $H_{\gamma Acp1}$ with NH_{Ach}, $H_{\gamma Acp2}$ with NH_{Aga}, $H_{\epsilon Aga}$ with NH_{Ach} and one cyclohexyl proton of Ach, and $H_{\delta Aga}$ with other cyclohexyl proton of Ach; all this cross-peaks suggest the formation of dimer **$D2_A$**. Protons in green and with wedged lines are those oriented towards peptide interface, while those in blue (hashed lines) are the solvent oriented protons.

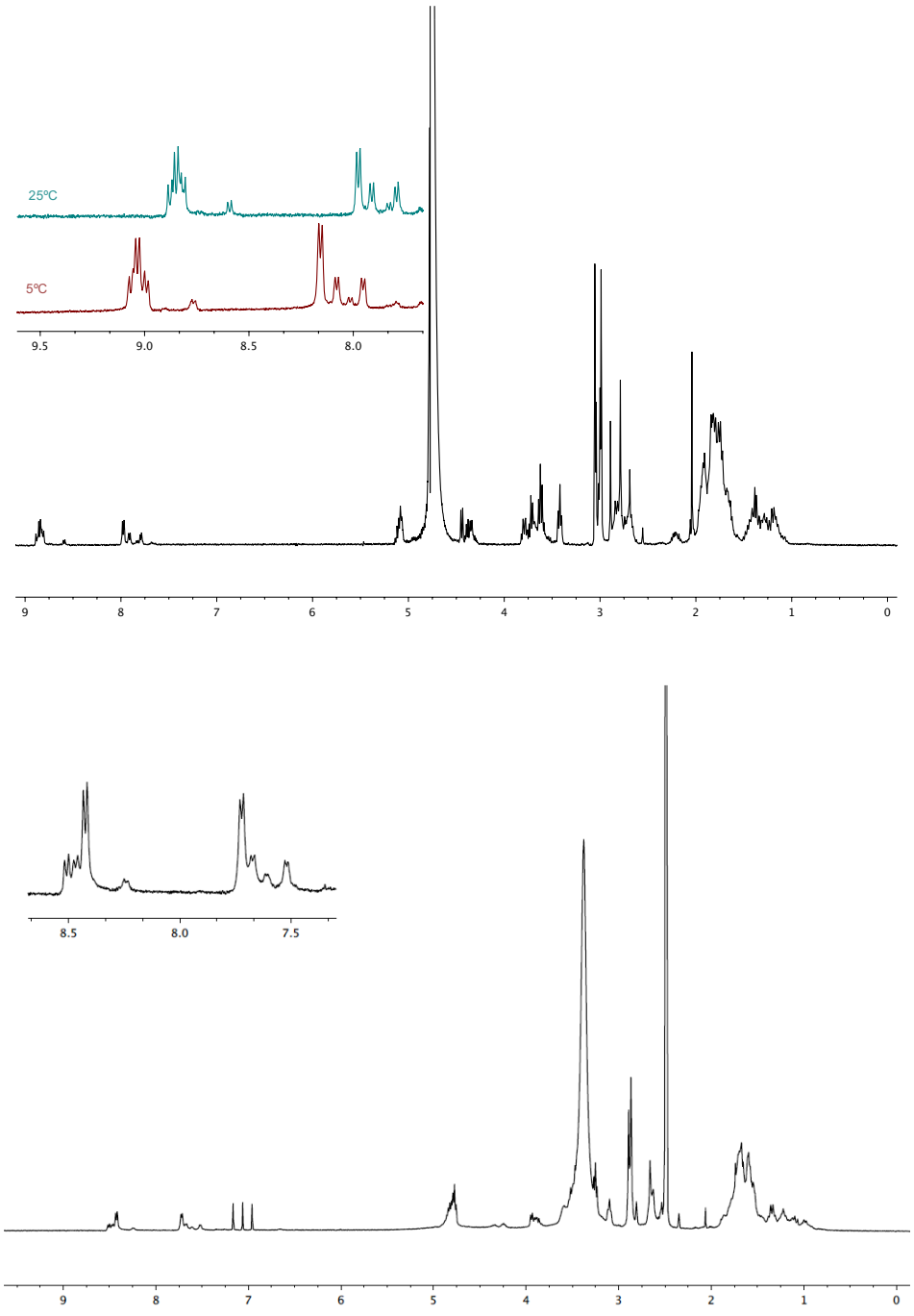


Figure 3SI: ^{1}H NMR spectrum of **γ-CP2** in H_2O (top) and DMSO (bottom) at 298K in which the peptide adopts several conformations. In the inset, the spectra of the amide region (9.60-7.80 ppm) at two temperatures (278-298K) are showed, confirming the existence of at least four different conformations.[1]

[1] The chemical shift of water (HOD) at different temperatures was calculated using the equation:

$$\delta = 5.060 - 0.0122T + (2.11 \times 10^{-5})T^2$$

For further information, see reference Gottlieb, H. E.; Kotlyar, V.; Nudelman, A.; *J. Org. Chem.,* **1997**, 62, 7512–7515.

1. Materials and Methods.

General:

1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo-[4,5-*b*]pyridinium hexafluorophosphate 3-oxide (HATU), 1-[bis(dimethylamino)methylene]-1*H*-benzotriazolium hexafluorophosphate 3-oxide (HBTU), 1-[bis(dimethylamino)methylene]-1*H*-benzotriazolium tetrafluoroborate 3-oxide (TBTU),[2] *N*,*N*´-Diisopropylcarbodiimide (DIC), *N,N'*-diciclohexilcarbodiimida (DCC), and 4-Dimethylaminopyridine (DMAP), alpha-aminoacids were purchased from Novabiochem, Applied Biosystems, Aldrich or from Global Sales Manager, GL Biochem (Shanghai) Ltd, China. All reagents and solvents were used as received unless otherwise noted. CH_2Cl_2 and DIEA to be used as reaction solvents were distilled from CaH_2 over argon immediately prior to use. Tetrahydrofurane (THF) was dried and distilled over sodium/benzophenone.[3] Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F_{254} plates. Compounds, which were not UV active, were visualized by dipping the plates in a nynhidrin solution and heating. Silica gel flash chromatography was performed using E. Merck silica gel (type 60SDS, 230-400 mesh). Solvent mixtures for chromatography are re-ported as v/v ratios. HPLC purification was carried out on phenomenex Luna 5u Silica 100 Angstroms column with CH_2Cl_2/MeOH gradients between 100 and 85:15 or on phenomenex Luna 5u C-18 100 Angstroms with H_2O (0.1% TFA)/CH_3CN (0.1% TFA) gradients between 5:95 and 75:25. **^{1}H NMR** and **^{13}C NMR** spectra were recorded on Bruker AMX 500 MHz, Varian Mercury 300 MHz or Bruker WM 250 MHz spectrometers. Chemical shifts (d) were reported in parts per million (ppm) relative to tetramethylsilane (d=0.00 ppm) or by the deuterium solvent. ^{1}H NMR splitting patterns are designated as singlet (s), doublet (d), triplet (t), or quartet (q). All first-order splitting patterns were assigned on the basis of the appearance of the multiplet. Splitting patterns that could not be easily interpreted are designated as multiplet (m) or broad (br). **^{1}H NMR Assignments of Cyclic Peptides (CPs)**. The signals of the ^{1}H NMR spectra of the peptides in $CDCl_3$ were identified from the corresponding double-quantum-filled 2D COSY, TOCSY and/or NOESY and ROESY spectra acquired at concentration and temperature indicated (Mixing times for NOESY and/or ROESY -between 250 and 1000 ms- were not optimized). Carbon resonances were assigned using distortionless enhancement by polarization transfer (DEPT) spectra obtained with phase angles of 135. Fast Atom Bombardement (FAB) mass spectra were recorded on a Micromass Autospec mass spectrometer. Electrospray (ESI) mass spectra were recorded on a Bruker BIOTOF II mass spectrometer. Mass Spectrometry of Laser Desorption/Ionization-Time of Flight (MALDI-TOF) was obtained on a Bruker Autoflex mass

[2] L. A. Carpino, et all., *Angew. Chem. Int. Ed.,* 2002, **41**, 441–445.
[3] (a) H.C. Brown, "*Organic Synthesis via Boranes"*, Ed. John Wiley & Sons, 1975. (b) Perrin, D. D.; Armarego, W. I. F. *"Purification of Laboratory Chemicals"*, Ed. Pergamon Press, 1988.

spectrometer. FTIR measurements were made on a JASCO FT/IR-400 spectrophotometer placing the sample on a CaF_2 pellet.

Peptide Synthesis:

Synthesis of amino acids and lineal-dipeptides, linear-tetrapeptides and linear-hexapeptides were prepared following the synthetic strategy previously described.[4]

Boc-*D*-γ-^{Me}*N*-Acp-OAll. A solution of Boc-*D*-γ-^{Me}*N*-Acp-OH (1.00 g, 4.12 mmol) in dry 2-propen-1-ol (24.0 mL) was treated with DCC (1.70 g, 8.24 mmol) and DMAP (100.0 mg, 0.82 mmol). After stirring for 4 h at rt, the reaction mixture was evaporated to dryness. The resulting residue was dissolved in CH_2Cl_2 (50 mL), and washed with aqueous HCl (5%, 3 x 15 mL) and aqueous saturated $NaHCO_3$ (3 x 15 mL). The organic layer was dried with Na_2SO_4, filtered, concentrated under reduced pressure, and the resulting crude material was purified by flash chromatography (10-30% AcOEt/Hexane) to give 986 mg of the title compound. [Yellow oil, 84%, R_f=0.85 (50% AcOEt/Hexanes)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 5.86 (ddt, *J* = 17.2, 10.4 and 5.7 Hz, 1H), 5.25 (dq, *J* = 17.2 and 1.5 Hz, 1H), 5.18 (dq, *J* = 10.4 and 1.3 Hz, 1H), 4.52 (brd, *J* = 5.4 Hz, 3H), 2.76 (s, 3H), 1.41 (s, 9H). **^{13}C NMR** ($CDCl_3$, 62.9 MHz, δ): 179.9 (CO), 156.2 (CO), 132.6 (CH), 118.4 (CH_2), 79.7 (C), 65.5 (CH_2), 56.0 (CH), 42.6 (CH_2), 41.6 (CH), 32.2 (CH_3), 29.2 (CH_2), 28.7 (CH_3), 27.6 (CH_2). **MS (ESI)** [m/z(%)]: 306.1 ([MNa]$^+$, 20). **HRMS (ESI) [MNa]$^+$ calculated** for $C_{15}H_{25}NO_4Na$: 306.1676, **found**: 306.1664.

1,2,3,4-Tetra-*O*-acetyl-β-D-glucuronic Acid Methyl Ester.[5] Glucuronic acid (4.0 g, 20.6 mmol) was suspended in acetic anhydride (50 mL) and stirred at 0 °C and then iodine (28 mg, 1.1 mmol) was slowly added. After stirring for 2 h at 0 °C and 1 h at rt, and the cooled down to 0 °C and then treated (drop wise) with dry MeOH (20 mL). The resulting mixture was stirred at rt for 18 h and concentrated to dryness. The residue was dissolved in CH_2Cl_2 (70 mL), washed with aqueous $Na_2S_2O_3$ (1 M, 3 x 50 mL) and brine (50 mL). The organic layer was dried with Na_2SO_4, filtered, concentrated under reduced pressure to give a white solid. The solid was extracted with a mixture of Et_2O/$CHCl_3$/hexanes (1:1:1, 50 mL) and the solution was concentrated under vacuum. The resulting foam was dissolved in Et_2O (100 mL), filtered and the resulting crude material was crystallized from EtOAc/hexanes (1:1) to give 6.4 g of the tetraacetylated glucuronic acid. [White solid, 85%, R_f = 0.64 (MeOH)]. **^{1}H NMR**

[4] (a) M. Amorín, L. Castedo, J. R. Granja, *J. Am. Chem. Soc.,* 2003, **125**, 2844–2845. (b) M. Amorín, V. Villaverde, L. Castedo, J. R. Granja, *J. Drug Del. Sci. Tech.,* 2005, **15**, 87–92. (c) R. J. Brea, M. Amorín, L. Castedo, J. R. Granja, *Angew. Chem. Int. Ed.,* 2005, **44**, 5710–5713.

[5] J. P. Malkinson, R. A. Falconer and I. Toth, *J. Org. Chem.* 2000, **65**, 5249–5252.

($CDCl_3$, 250 MHz, δ): 6.33 (brs, 1H), 5.71 (d, *J* = 7.7 Hz, 1H), 5.30-5.20 (m, 2H), 5.13 (m, 1H), 4.16 (m, 1H), 2.06 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H). **MS (ESI)** [m/z(%)]: 385.07 ($[MNa]^+$, 100).

2,3,4-Tri-*O*-acetyl-1-azido-1-deoxy-β-D-glucopyranuronic Acid.[5] To a solution of 1,2,3,4-Tetra-*O*-acetyl-β-D-glucuronic Acid Methyl Ester (1.0 g, 2.8 mmol) in CH_2Cl_2 (20 mL) was successively treated with trimethylsilyl azide (960 µL, 6.9 mmol) and tin tetrachloride in heptane (1 M, 1.40 mL, 1.4 mmol). The reaction was stirred for 18 h at rt under Ar. The solution was diluted with CH_2Cl_2 (15 mL) and washed with aqueous $Na_2S_2O_3$ (1 M, 3 x 15 mL), dried (Na_2SO_4), filtered, and concentrated to yield 780 mg of the title compound. [White foam, 82%, R_f = 0.59 (10% MeOH/CH_2Cl_2 with 1% AcOH)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 9.07 (brs, 1H), 5.32-5.12 (m, 2H), 4.92 (t, *J* = 8.8 Hz, 1H), 4.75 (t, *J* = 8.7 Hz, 1H), 4.13 (t, *J* = 9.3 Hz, 1H), 2.03 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H). **MS (ESI)** [m/z(%)]: 368.06 ($[MNa]^+$, 100), 385.07 ($[MK]^+$, 42).

Boc-*L*-γ-Ach-*D*-γ-^{Me}*N*-Acp-OFm (1). A solution of Boc-*D*-γ-^{Me}*N*-Acp-OFm[3c] (250.0 mg, 0.59 mmol) in a TFA/CH_2Cl_2 mixture (1:1, 6.0 mL) was stirred at rt for 30 min. After removal of the solvent *under vacuum*, the residue was dried under high vacuum for 3 h. The resulting TFA salt was dissolved under Argon in dry CH_2Cl_2 (6.0 mL) and Boc-*L*-γ-Ach-OH (158.0 mg, 0.65 mmol), HATU (270.0 mg, 0.71 mmol), and DIEA (610 µL, 3.54 mmol) were successively added. After 1 h stirring at rt, the solution was washed with aqueous HCl (5%, 3 x 5 mL) and aqueous saturated $NaHCO_3$ (3 x 5 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure, providing a yellow oil that was purified by flash chromatography (20-40% EtAcO/hexanes) to give 267.0 mg of the dipeptide **1**. [White foam, 83%, R_f = 0.55 (5% MeOH/CH_2Cl_2)]. **^{1}H NMR** ($CDCl_3$, 400 MHz, δ): 7.80 (d, *J* = 7.5 Hz, 2H), 7.62 (d, *J* = 7.4 Hz, 2H), 7.34 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 5.03 (m, 1H), 4.63-4.47 (m, 4H), 4.24 (t, *J* = 6.2 Hz, 2H), 3.53 (m, 1H), 2.85 (s, 3H), 1.47 (s, 9H). **^{13}C NMR** ($CDCl_3$, 62.9 MHz, δ): 175.9 (CO), 175.2 (CO), 155.3 (CO), 143.8 (C), 141.5 (C), 127.9 (CH), 127.2 (CH), 125.0 (CH), 120.1 (CH), 79.3 (C), 66.1 (CH_2), 57.5 (CH), 53.7 (CH), 49.2 (CH), 47.1 (CH), 41.7 (CH), 40.4 (CH), 39.9 (CH) 35.9 (CH_2) 33.0 (CH_2) 31.4 (CH_2), 29.3 (CH_3), 29.0 (CH_2), 28.5 (CH_3), 28.5 (CH_2), 28.3 (CH_2), 27.7 (CH_2), 27.2 (CH_2). **MS (FAB$^+$)** [m/z(%)]: 569.3 ($[MNa]^+$, 5), 547.3 ($[MH]^+$, 39), 447.2 ($[MH\text{-}Boc]^+$, 100). **HRMS (ESI) calculated** for $C_{33}H_{43}N_2O_5$: 547.3172, **found**: 547.3165.

N_3-*D*-γ-$(Ac)_3$Aga-*D*-γ-^{Me}*N*-Acp-OAll (3). The mentioned compound was prepared in the same way as **1** starting from Boc-*D*-γ-^{Me}*N*-Acp-OFm[3c] (300.0 mg, 0.71 mmol) and 2,3,4-Tri-*O*-acetyl-1-azido-1-deoxy-β-D-glucopyranuronic acid (287.0 mg, 0.78 mmol) to yield 290 mg of dipeptide **3**. [White foam, 80%, R_f = 0.46 (2% MeOH in CH_2Cl_2)]. **^{1}H NMR** ($CDCl_3$, 250 MHz, δ): 5.85 (m, 1H), 5.45 (t, *J* = 9.5Hz, 1H), 5.32-5.05 (m, 3H), 4.92 (td, *J* = 9.1 and 4.0 Hz, 1H), 4.67 (dd, *J*= 8.6 and 6.7 Hz, 1H), 4.53 (t, *J* = 5.0 Hz,

2H), 4.41-4.21 (m, 2H), 2.92-2,79 (2s, 3H), 2.01 (s, 3H), 1.95 (s, 3H), 1.90 (s, 3H). **^{13}C NMR** ($CDCl_3$, 62.9 MHz, δ): 175.8 (CO), 170.5 (CO), 169.3 (CO), 169.0 (CO), 164.8 (CO), 132.3 (CH), 118.5 (CH_2), 74.1 (CH), 73.7 (CH), 70.5 (CH), 69.2 (CH), 65.5 (CH_2), 55.0 (CH), 41.6 (CH), 32.7 (CH_2), 31.4 (CH_2), 29.3 (CH_3), 27.2 (CH_2), 20.8 (3xCH_3). **MS (ESI)** [m/z(%)]: 511.2 ([M]$^+$, 100)]. **HRMS (FAB+) calculated** for $C_{22}H_{31}N_4O_{10}$: 511.2040, **found**: 511.2053.

Boc-*L*-γ-Ach-*D*-γ-^{Me}*N*-Acp-*D*-γ-(Ac)$_3$Aga-*D*-γ-^{Me}*N*-Acp-OAll (4). A solution of the dipeptide Boc-*L*-γ-Ach-*D*-γ-^{Me}N-Acp-OFm (**1**) (250.0 mg, 0.46 mmol) in a mixture of piperidine and CH_2Cl_2 (1:4, 4.5 mL) was stirred at rt for 30 minutes, then the solvent was removed *under vacuum* and the residue was dissolved in CH_2Cl_2 (10 mL). This solution was washed with aqueous HCl (5%, 3 x 10 mL), dried over Na_2SO_4, filtered and concentrated, to give Boc-*L*-γ-Ach-*D*-γ-^{Me}*N*-Acp-OH (**2**), which was used without further purification. Dipeptide N_3-*D*-γ-(Ac)$_3$Aga-*D*-γ-^{Me}*N*-Acp-OAll (**3**) (256.5 mg, 0.50 mmol) was dissolved in dry THF (5 mL) and stirred at -55ºC. Tri-*n*-butylphosphine (140 µL, 0.55 mmol) was added and the solution was stirred during 30 min at the same temperature. A solution of Boc-*L*-γ-Ach-*D*-γ-^{Me}*N*-Acp-OH (**2**), DIC (140 µL, 0.91 mmol), and HOBt (123.5 mg, 0.91 mmol) in THF (5 mL) was added and the reaction mixture was stirred while allowed to reach room temperature overnight. The reaction mixture was concentrated under reduced pressure, dissolved in CH_2Cl_2 (5.0 mL) and washed with aqueous HCl (5%, 3 x 10 mL) and aqueous saturated $NaHCO_3$ (3 x 10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (1-3% MeOH/CH_2Cl_2) to produce 230.0 mg of **4**. [Pale yellow foam, 60%, R_f = 0.33 (5% MeOH/CH_2Cl_2)]. **MS (FAB$^+$)** [m/z(%)]: 835.5 ([MH]$^+$, 1), 735.5 ([MH-Boc]$^+$, 6). **HRMS (FAB+) calculated** for $C_{41}H_{63}N_4O_{14}$: 835.4341, **found**: 835.4327.

Boc-[*L*-γ-Ach-*D*-γ-^{Me}*N*-Acp-*D*-γ-(Ac)$_3$Aga-*D*-γ-^{Me}*N*-Acp-]$_2$OAll (5). A solution of the tetrapeptide **4** (240.0 mg, 0.240 mmol) in CH_2Cl_2 (20 mL) was successively treated with $Pd(OAc)_2$ (9.7 mg, 44 µmol), N-methylmorpholine (320 µL, 2.90 mmol), $PhSiH_3$ (360 µL, 2.90 mmol), and PPh_3 (34.0 mg, 0.13 mmol). After stirring for 1 h at rt, the mixture was washed with aqueous HCl (5%, 3 x 10 mL), dried over Na_2SO_3, filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography (3-8% MeOH/CH_2Cl_2) to obtain 175.0 mg of the peptide Boc-*L*-γ-Ach-*D*-γ-^{Me}*N*-Acp-*D*-γ-(Ac)$_3$Aga-*D*-γ-^{Me}*N*-Acp-OH [76%, *Rf* = 0.22 (5% MeOH/CH_2Cl_2)].

A solution of Boc-*L*-γ-Ach-*D*-γ-^{Me}*N*-Acp-*D*-γ-Aga-*D*-γ-^{Me}*N*-Acp-OAll (175.0 mg, 0.21 mmol) in a mixture of TFA in CH_2Cl_2 (1:4, 2.5 mL) was stirred at rt for 60 min. After removal of the solvent, the residue was dried under high vacuum for 3 h. The resulting residue was dissolved in dry CH_2Cl_2 (2.5 mL) and treated with previously prepared Boc-*L*-γ-Ach-*D*-γ-^{Me}N-Acp-*D*-γ-(Ac)$_3$Aga-*D*-γ-^{Me}*N*-Acp-OH (175.0 mg, 0.21 mmol), HATU (96.0 mg, 0.25 mmol) and DIEA (210 µL, 1.20 mmol). After 90 min stirring at rt, the

mixture was washed with aqueous HCl (5%, 3 x 5 mL), dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified by flash chromatography (1-7% of MeOH/CH_2Cl_2) to give 175.0 mg of the peptide **5**. [Pale yellow foam, 70%, R_f = 0.30 (5% MeOH/CH_2Cl_2)]. **MS (FAB$^+$)** [m/z (%)]: 1512.8 ([MH]$^+$, 1), 756.9 ([MH]$^{2+}$, 15), 756.4 ([MH]$^{2+}$, 18), 706.9 ([MH-Boc]$^{2+}$, 25). **HRMS (ESI) calculated** for $C_{74}H_{112}N_8O_{25}$ ([MH]$^{2+}$): 756.3864, **found**: 756.3861.

***c*-{[*L*-γ-Ach-*D*-γ-^{Me}*N*-Acp-*D*-γ-(Ac)$_3$Aga-*D*-γ-^{Me}*N*-Acp-]$_2$} (γ-CP1).** The octapeptide **5** (90.0 mg, 59.4 μmol) was dissolved in dry THF (1.0 mL) and then treated with $Pd(PPh_3)_4$ (7.0 mg, 6.0 μmol) and 4-methylmorpholine (50 μL, 0.600 mmol). After 2 h, the solvent was evaporated, and the residue was dissolved in CH_2Cl_2 (5 mL), washed with aqueous HCl (5%, 3 x 5 mL), dried over Na_2SO_4, filtered and concentrated to dryness. The resulting residue was purified by flash chromatography (2-6% MeOH/CH_2Cl_2) to obtain 74.0 mg of Boc-[*L*-γ-Ach-*D*-γ-^{Me}*N*-Acp-*D*-γ-(Ac)$_3$Aga-*D*-γ-^{Me}*N*-Acp-]$_2$OH [85%, *Rf* = 0.20 (5% MeOH/CH_2Cl_2)]. The resulting C-unprotected octapeptide (70.0 mg, 47.5 μmol) was dissolved in a mixture of TFA and CH_2Cl_2 (1:4, 2.0 mL) and stirred for 1 h at rt. After removal of the solvent under reduced pressure, the residue was dried under high vacuum for 3 h and used without further purification. The resulting unprotected linear peptide was dissolved in CH_2Cl_2 (56 mL) and treated with TBTU (22.0 mg, 68.5 μmol), followed by dropwise addition of DIEA (60 μL, 0.336 mmol). After 12 h, the solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (15 mL), washed with aqueous HCl (5%, 3 x 5 mL), dried over Na_2SO_4, filtered and concentrated to dryness. The crude was purified by HPLC (Phenomenex Luna 5μ silica, 5-10% MeOH/CH_2Cl_2), to afford 16.0 mg of **γ-CP1** as a white solid (25%). **^{1}H NMR** ($CDCl_3$, 500 MHz, δ): 9.01 (d, *J* = 9.8 Hz, 2H, NH_{Aga}), 7.47 (d, *J* = 7.5 Hz, 2H, NH_{Ach}), 5.45 (m, 4H, $H_{\gamma Aga}$ and $H_{\varphi Aga}$), 5.32 (t, *J* = 9.4 Hz, 2H, $H_{\varepsilon Aga}$), 5.21 (t, *J* = 9.4 Hz, 2H, $H_{\delta Aga}$), 4.76 (m, 4H, $H_{\gamma Acp}$), 4.48 (d, *J* = 9.7 Hz, 2H, $H_{\alpha Aga}$), 3.95 (m, 2H, $H_{\gamma Ach}$), 3.01 (s, 6H, NMe), 2.94 (s, 6H, NMe), 2.82 (m, 2H, $H_{\alpha Ach}$), 2.69 (m, 4H, $H_{\alpha Acp}$), 1.98 (s, 6H, AcO), 1.97 (s, 6H, AcO), and 1.95 (s, 6H, AcO). **^{13}C NMR** ($CDCl_3$, 126.0 MHz, δ): 175.9 (CO), 175.5 (CO), 173.9 (CO), 170.2 (CO), 169.3 (CO), 168.7 (CO), 165.9 (CO), 78.3 (CH), 73.5 (CH), 72.0 (CH), 70.7 (CH), 70.6 (C), 55.1 (CH), 54.4 (CH), 46.7 (CH), 43.1 (CH), 42.7 (CH), 40.4 (CH), 36.0 (CH_2), 32.3 (CH_2), 31.1 (CH_2), 29.7 (CH_2), 29.4 (CH_3), 29.3 (CH_3), 28.2 (CH_2), 28.1 (CH_2), 27.9 (CH_2), 27.6 (CH_2), 26.3 (CH_2), 24.8 (CH_2), 20.6 and 20.5 (3xCH_3). **EM (ESI)** [m/z (%)]: 677.5 ([MH]$^{2+}$, 100), 1354.8 ([MH]$^+$, 5), 1375.6 ([MH+Na]$^+$, 4). **HRMS (ESI) calculated** for $C_{68}H_{97}N_8O_{22}$**:** 1353.6717, **found**: 1353.6713. **FTIR** (293 K, $CHCl_3$): 3321 and 3276 (amide A), 2943, 2886, 1757, 1639 and 1626 (amide I), 1534 cm^{-1} (amide II$_{II}$).

***c*-[(*L*-γ-Ach-*D*-γ-^{Me}*N*-Acp-*D*-γ-Aga-*D*-γ-^{Me}*N*-Acp-)$_2$] (γ-CP2).** The cyclic peptide **γ-CP1** (3.0 mg, 2.2 μmol) was dissolved in MeOH (400 μL) and commercial solution of aqueous NH_3 (100 μL) was added.

After 1 h stirring, the solvent was removed under reduced pressure and the residue was purified by RP-HPLC [Phenomenex Luna 5µ C18, 5-40% ACN/H_2O (0.1%TFA)] to afford 2.2 mg of wished cyclic peptide as a white solid (90%). **^{1}H NMR** ($CDCl_3$/$(CD_3)_2SO$ (7:3), 500 MHz, δ): 8.53 (m, 2H, NH_{Aga}), 7.90 (m, 2H, NH_{Ach}), 5.05 (m, 2H, $H_{\gamma Aga}$), 4.81 (brs, 6H, $H_{\gamma Acp2}$ and OH_{Aga}), 4.73 (brs, 2H, $H_{\gamma Acp1}$), 4.61 (brs, 2H, OH_{Aga}), 4.21 (d, *J*=8.9 Hz, 2H $H_{\alpha Aga}$), 3.82 (s, 2H (H_{γ}Ach)), 3.62 (brs, 2H, $H_{\phi Aga}$), 3.46 (brs, 2H, $H_{\varepsilon Aga}$), 3.40 (s, 2H, H, $H_{\delta Aga}$), 2.95 (s, 6H, NMe), 2.88 (s, 6H, NMe), 2.86 (s, 2H, $H_{\alpha Acp1}$), 2.75 (s, 2H, $H_{\alpha Acp2}$), 2.65(s, 2H, $H_{\alpha Ach}$). **EM (ESI)** [m/z (%)]: 551.4 ($[MH]^{2+}$, 50), 1101.6 ($[MH]^+$, 8), 1123.5 ($[MNa]^+$, 4). **HRMS (ESI) calculated** for $C_{54}H_{85}N_8O_{16}$ $[MH]^+$: 1101.6084, **found**: 1101.6069. **FTIR** (293 K, $CHCl_3$): 3433 and 3311 (amide A), 2927, 2858, 1676, 1620 (amide I), 1545 cm^{-1} (amide II_{II}).

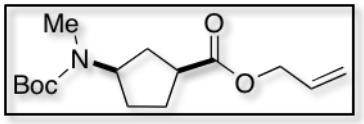
X-Ray Crystallographic Determination of D1$_A$

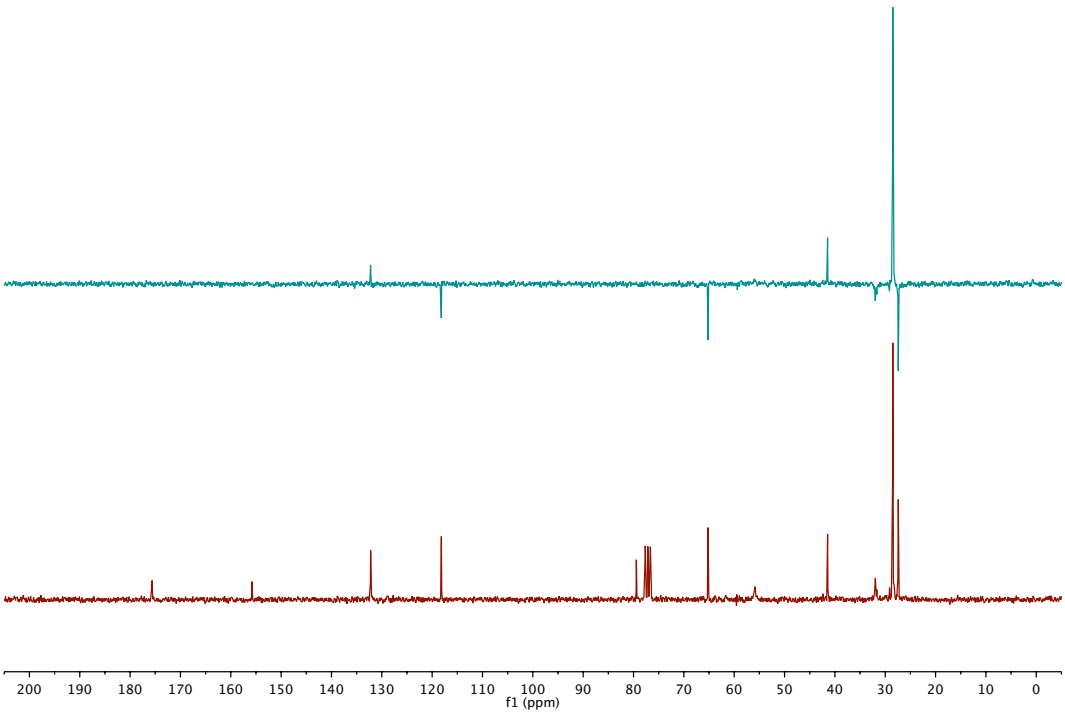
Preparation of single crystals for X-ray analysis: In a typical experiment, 3.0 mg of HPLC-purified **γ-*D1$_A$*** was dissolved in 1.0 mL of a mixture CH_2Cl_2/MeOH (95:5), and equilibrated by vapour-phase diffusion against 4.0 mL of hexanes. The corresponding dimer crystallized spontaneously within 2 days.

X-ray crystallographic analysis: data were collected at 100 K, using Bruker X8 Kappa APEXII CCD diffractometer using Mo *Kα* radiation and a graphite monocromator. All calculations were performed on a PC compatible computer using the programs: *SIR97* (Altomare *et al*., 1999), *SHELXL 97* (Sheldrick, 2008), *ORTEP-3* (Farrugia, 1997), Win-GX (Farrugia, 1999), *PLATON (SQUEEZE)* (Spek, 2001). Supplementary crystallographic data for ***γ-D1$_A$*** (CIF format) can be obtained free of charge from the journal.

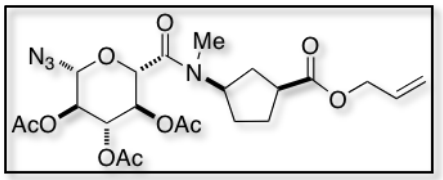
NMR SPECTRA

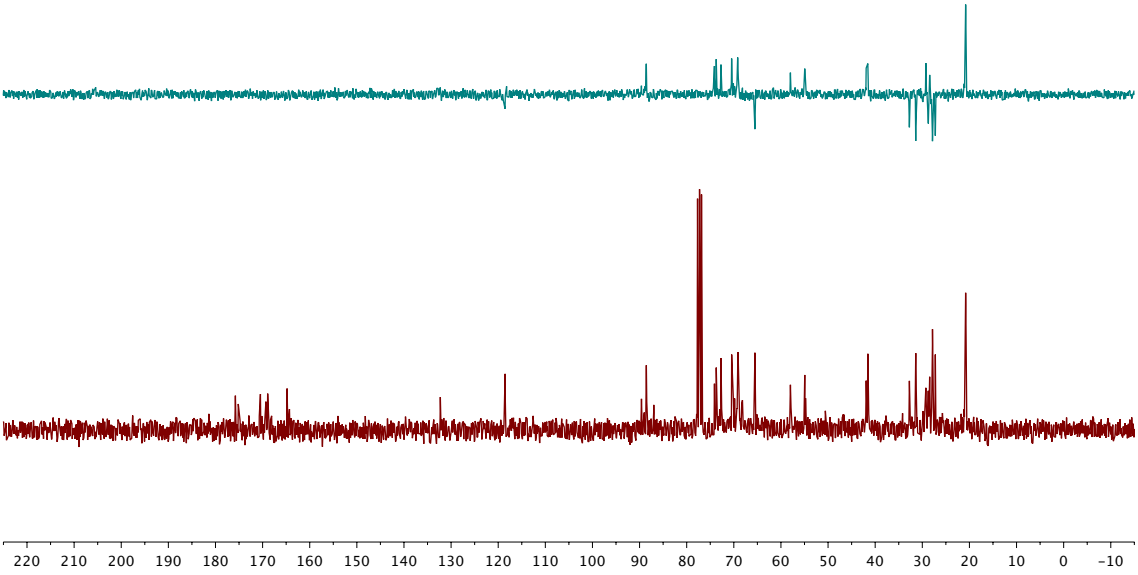
Boc-(*D*)-γ-^{Me}*N*-Acp-OAll



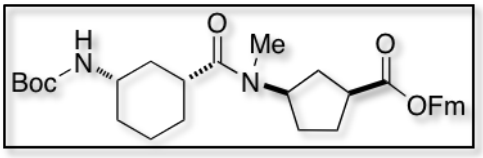


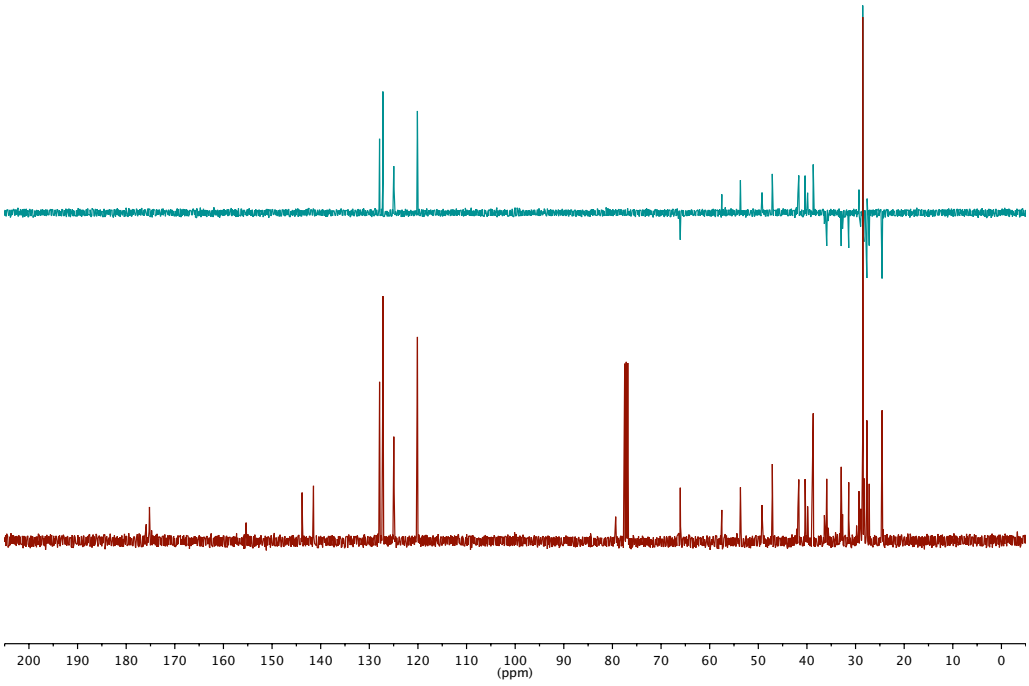
N_3-*D*-γ-Aga-*D*-γ-^{Me}*N*-Acp-OAll (1)





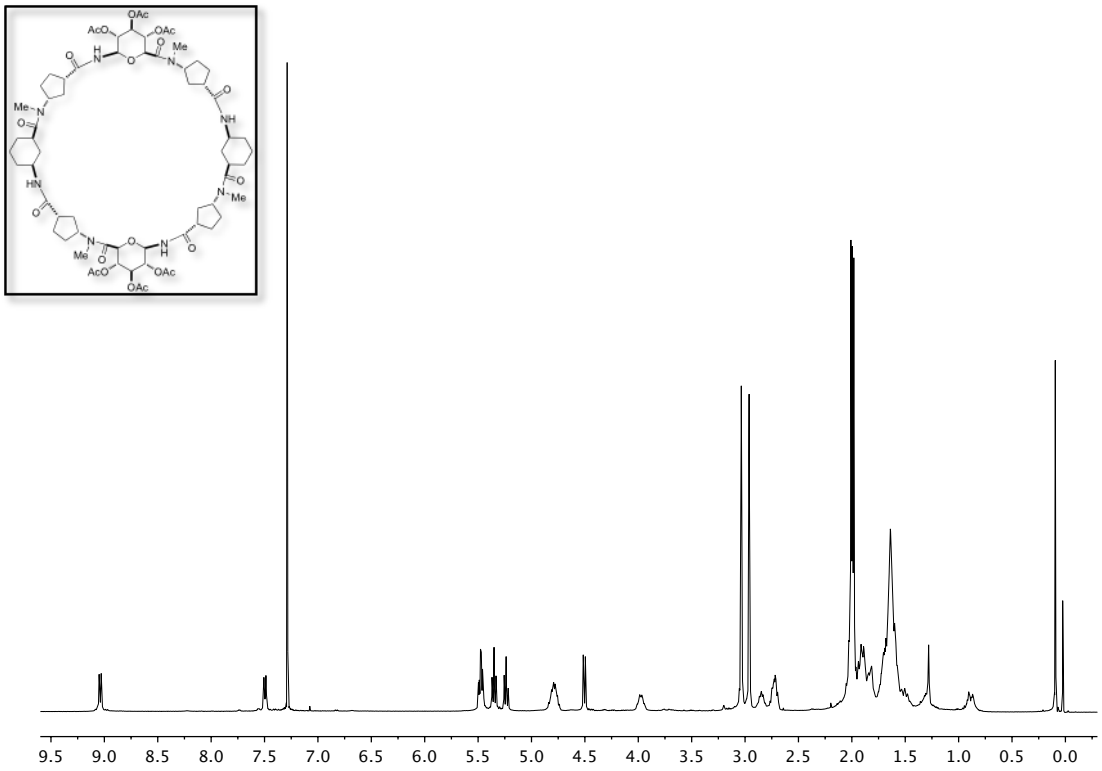
Boc-*L*-γ-Ach-*D*-γ-^{Me}*N*-Acp-OFm (2)



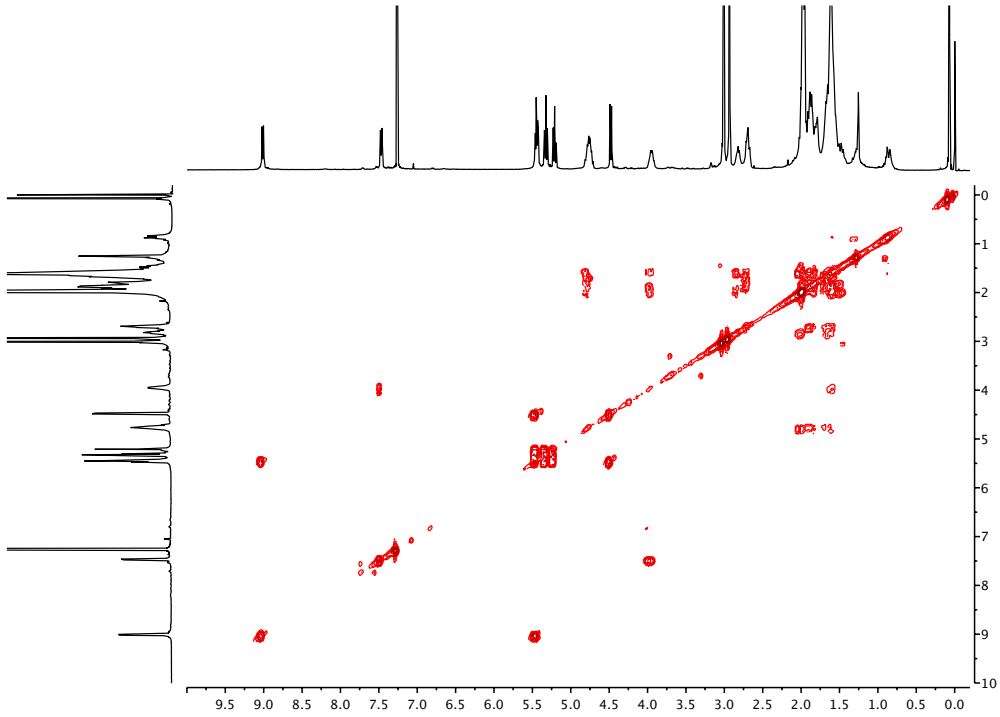


***c*-[(*L*-γ-Ach-*D*-γ-^{Me}*N*-Acp-*D*-γ-(Ac)$_3$Aga-*D*-γ-^{Me}*N*-Acp)$_2$-] (γ-CP1).**

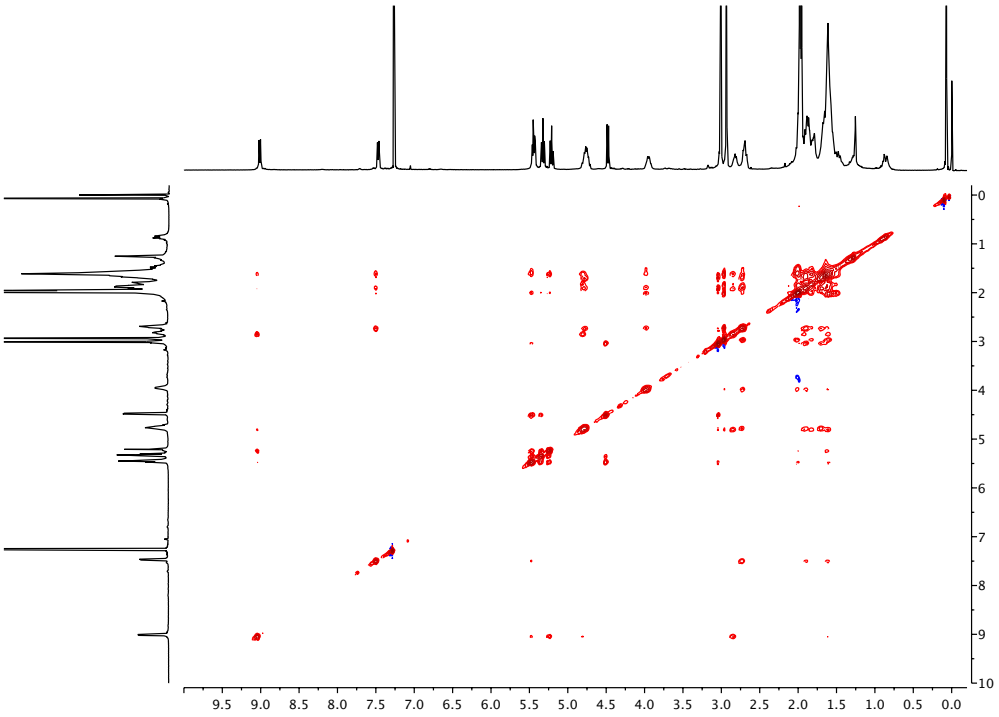
^{1}H NMR [2.8 mM, $CDCl_3$, 298K, 500.13 MHz].



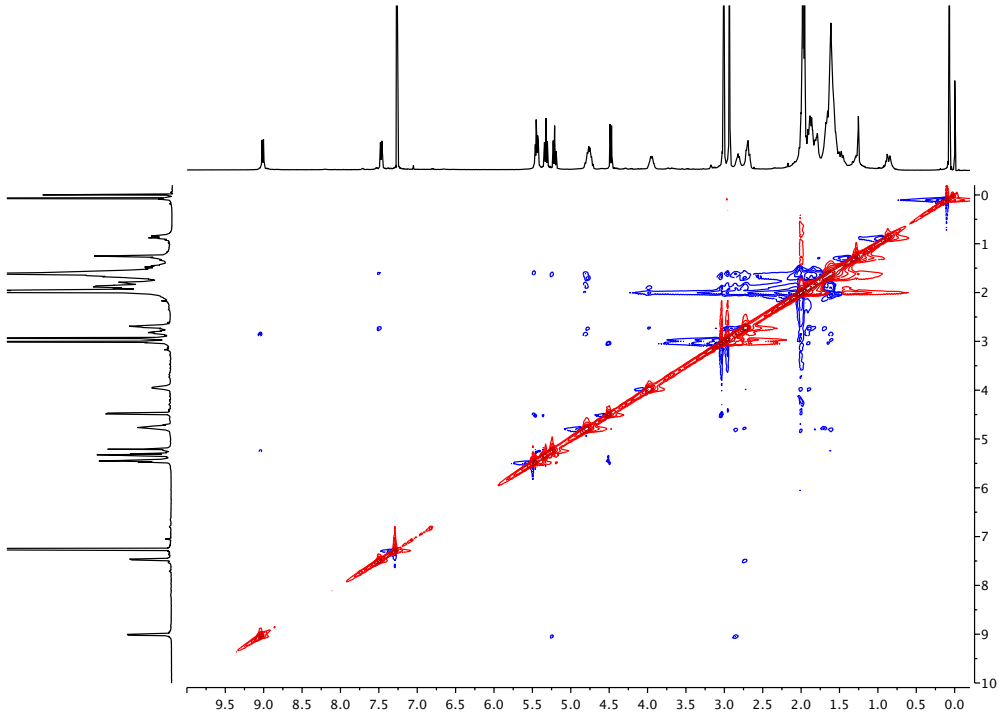
COSY [2.8 mM, $CDCl_3$, 298K, 500.13 MHz]



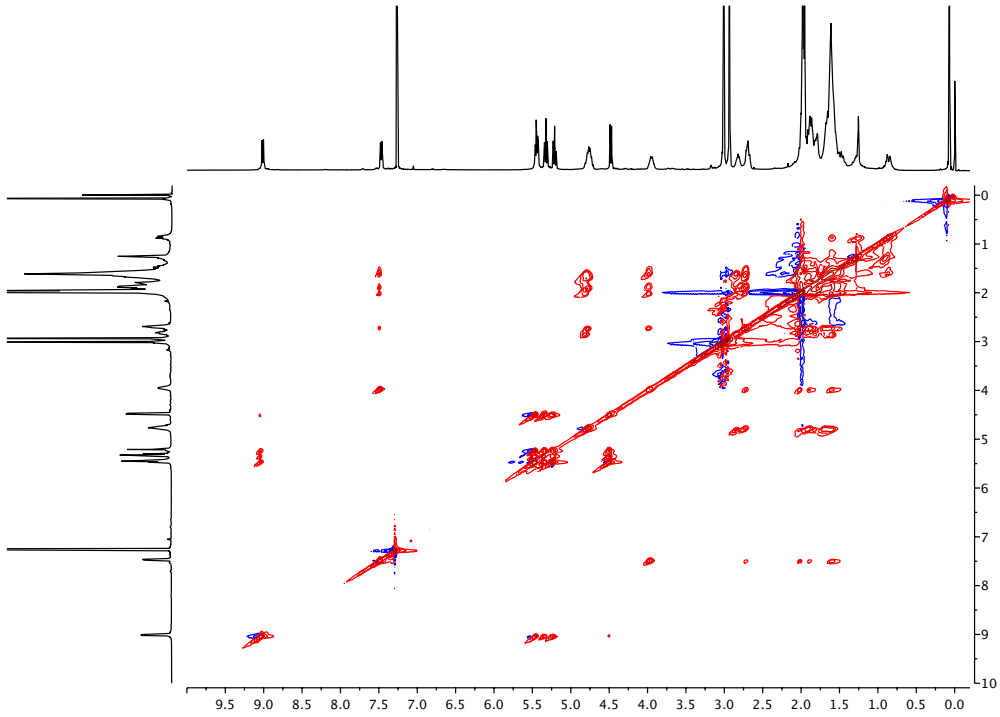
NOESY [2.8 mM, $CDCl_3$, 298K, 500.13 MHz]



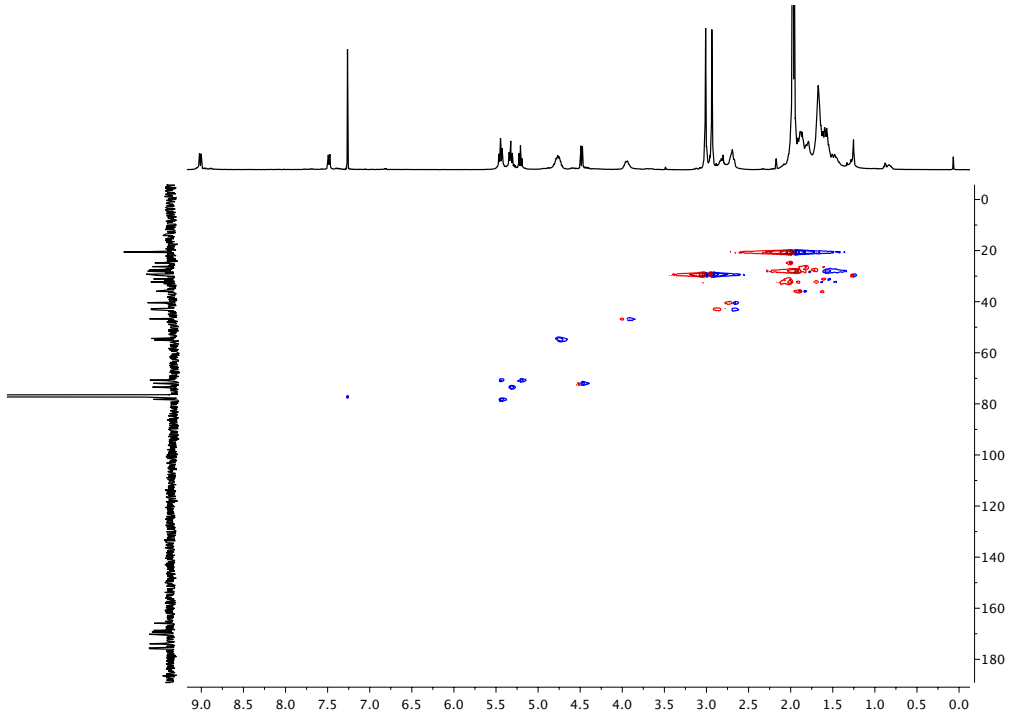
ROESY [2.8 mM, $CDCl_3$, 298K, 500.13 MHz]



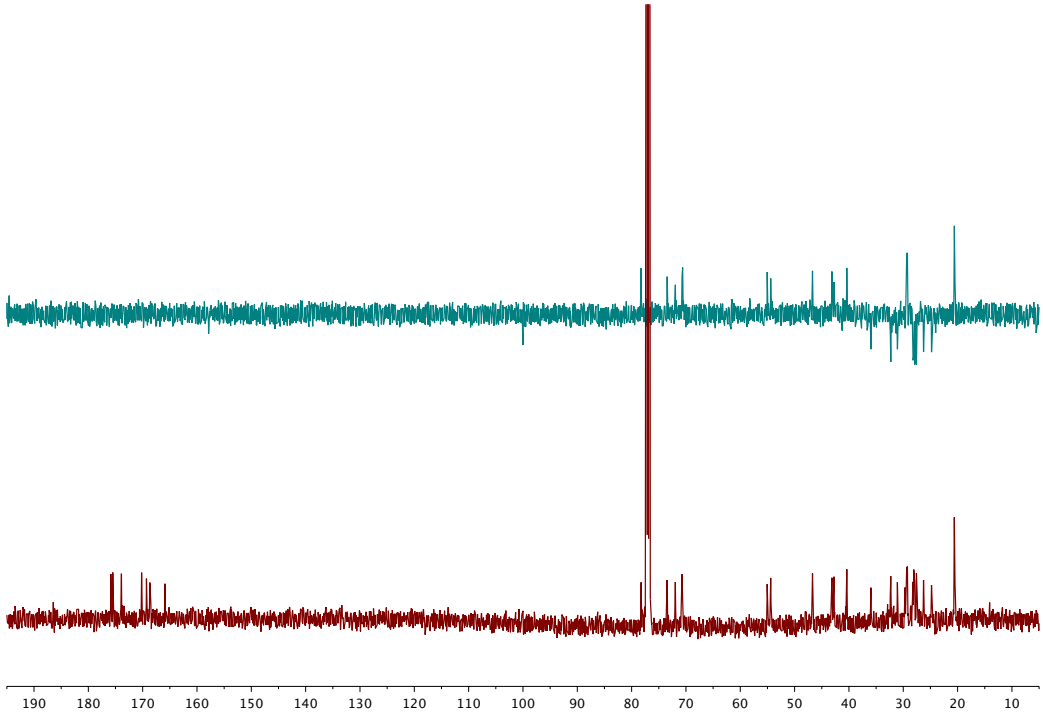
TOCSY [2.8 mM, $CDCl_3$, 298K, 500.13 MHz]



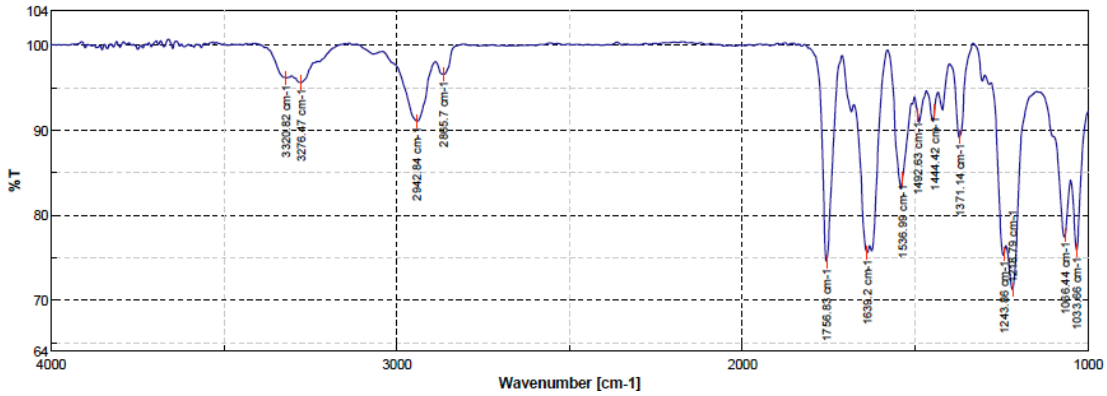
HSQC [2.8 mM, $CDCl_3$, 298K, 500.13 MHz]



^{13}C NMR and DEPT [2.8 mM, $CDCl_3$, 298K, 500.13 MHz]

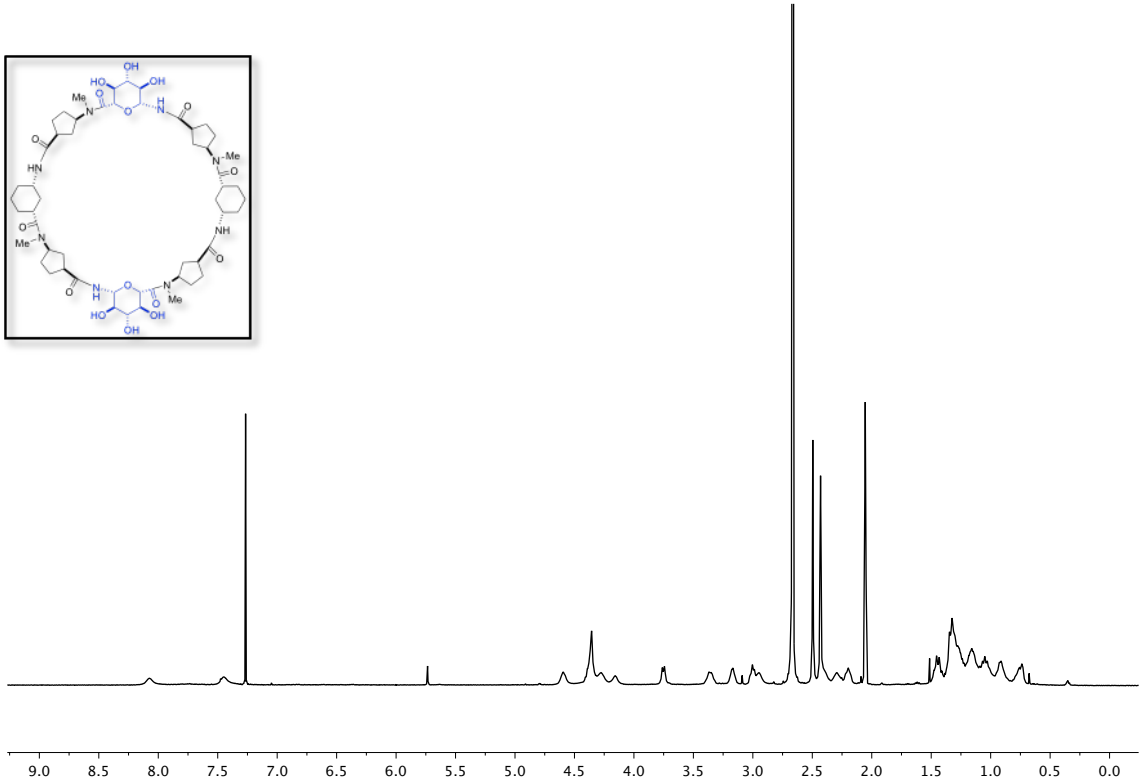


FT-IR [$CHCl_3$, 298K]

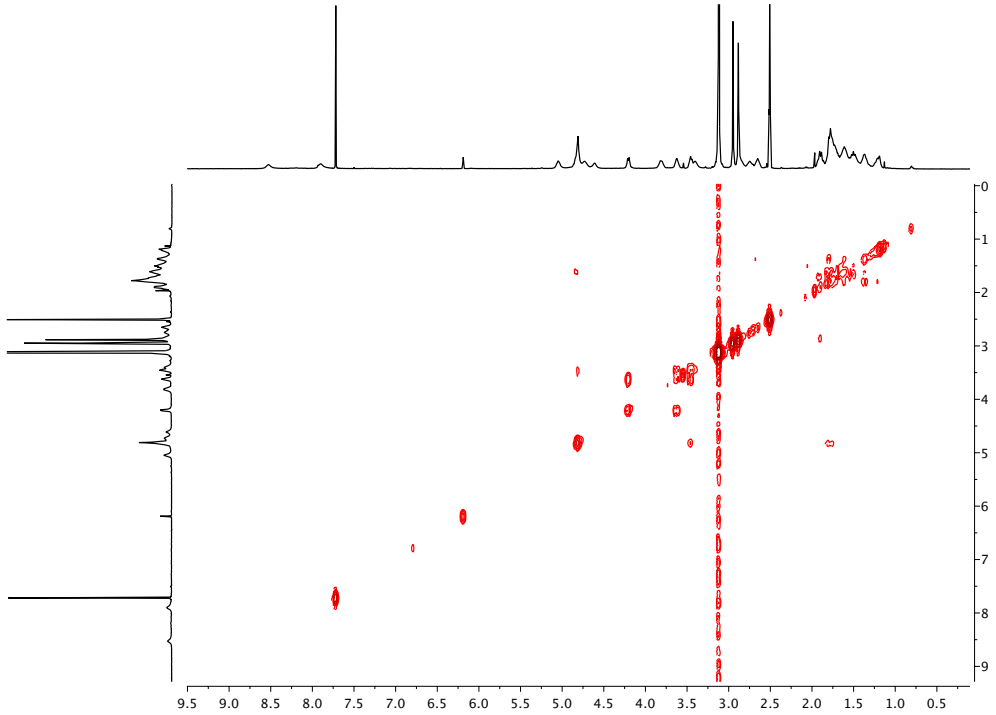


***c*-[(*L*-γ-Ach-*D*-γ-^{Me}*N*-Acp-*D*-γ-Aga-*D*-γ-^{Me}*N*-Acp)$_2$-] (γ-CP2).**

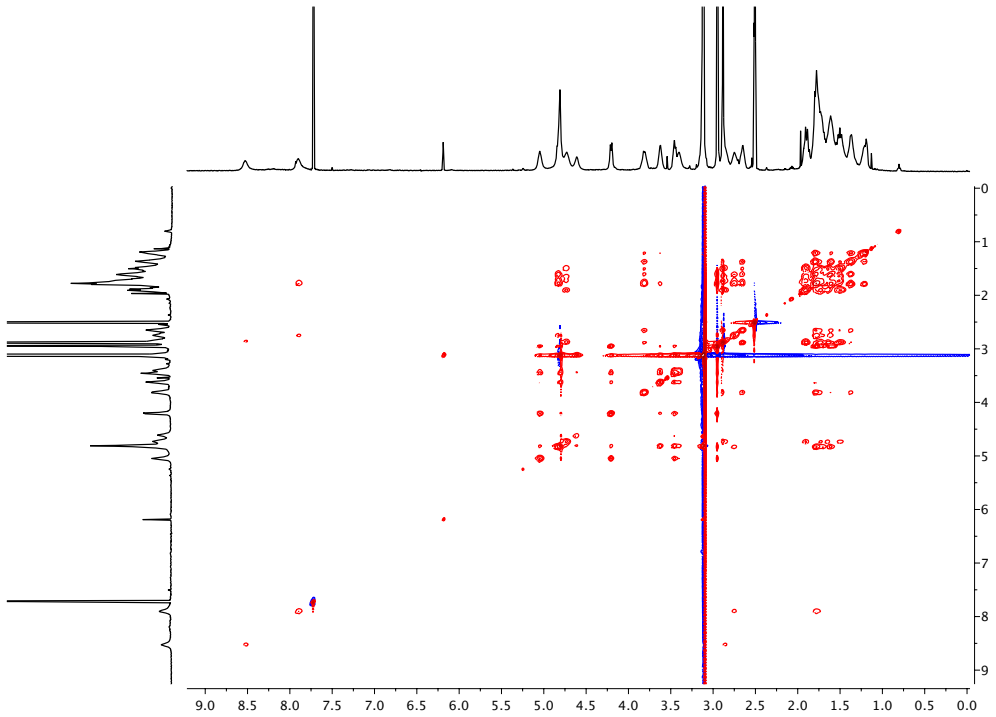
^{1}H NMR [9.0 mM, $CDCl_3$/$(CD_3)_2SO$ (7:3), 298K, 500.13 MHz].



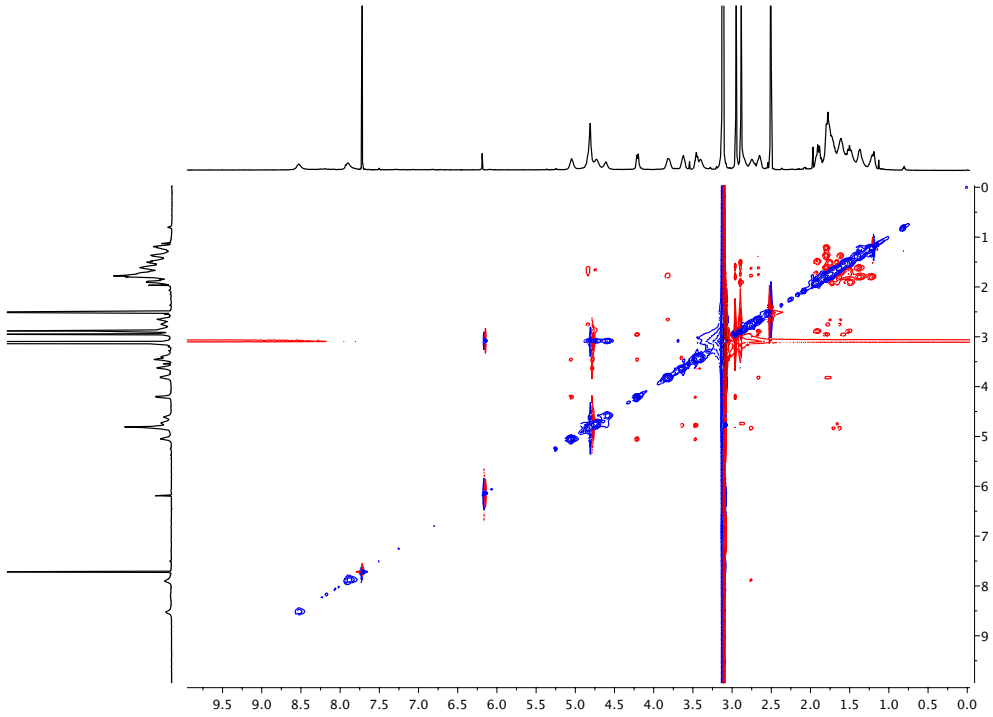
COSY [9.0 mM, $CDCl_3$/$(CD_3)_2SO$ (7:3), 298K, 500.13 MHz]



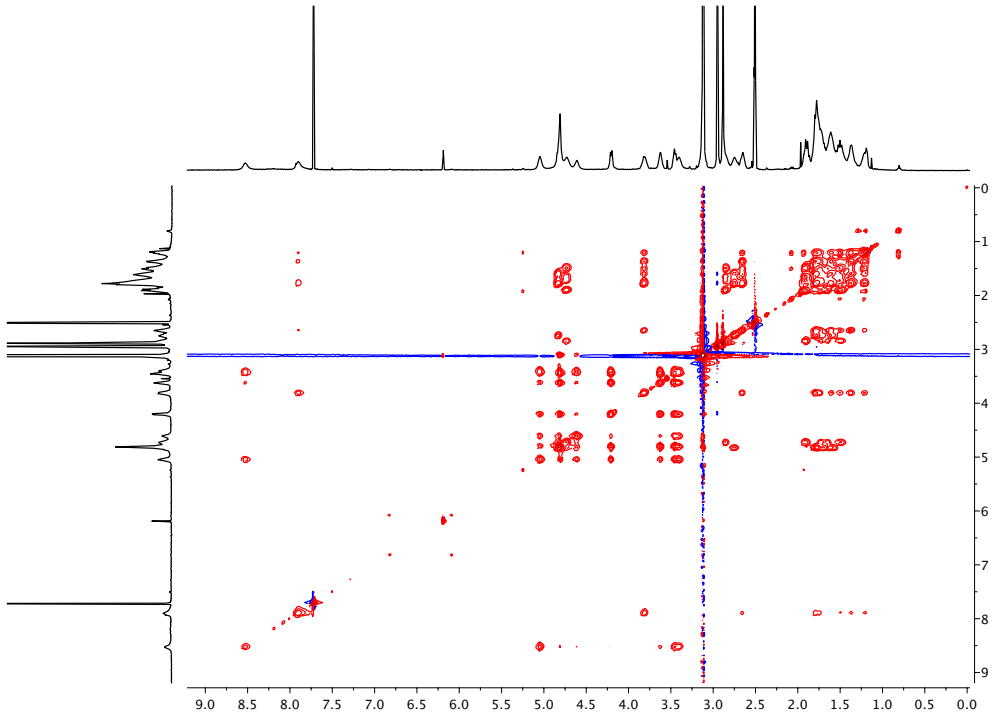
NOESY [9.0 mM, $CDCl_3$/$(CD_3)_2SO$ (7:3), 298K, 500.13 MHz]



ROESY [9.0 mM, $CDCl_3/(CD_3)_2SO$ (7:3), 298K, 500.13 MHz]



TOCSY [9.0 mM, $CDCl_3/(CD_3)_2SO$ (7:3), 298K, 500.13 MHz]



FT-IR [MeOH, 298K]